Lawrence Berkeley Laboratory NSCOR

NASA's Biomedical Critical Path Roadmap defines the carcinogenic risks of radiation exposure as one of only four Type I risks identified. A type I risk represents a demonstrated, serious problem with no countermeasure concepts, and may be a potential "show-stopper" for long duration spaceflight. Estimating the carcinogenic risks for different tissues in humans exposed to heavy ions is difficult at present. These ions contribute significantly to the dose and dose-equivalent received by astronauts during extended missions in low earth orbit (shuttle and ISS) and will be even more important for interplanetary excursions or lunar missions.

Space radiation environments are unlike any on earth. It is a significant challenge to estimate the biological consequences of human space flight because of the heterogeneous nature of the radiation fields, which include all charged particle species from protons through uranium at varying energies of up to tens of GeV/amu. High atomic mass (Z) and high energy (HZE) particle irradiation is of particular concern because the limited experimental data to date indicate the relative biological effect (RBE) for carcinogenesis for individual densely ionizing HZE particles is several-to-many fold greater than sparsely ionizing radiation like X-rays or g-radiation. Uncertainties arising from physical issues such as charged particle fragmentation in shielding and in human tissue and environmental issues such as prediction of solar particle events confound NASA's ability to predict the carcinogenic risks associated with space flight. These uncertainties are compounded further by our presently incomplete understanding of carcinogenesis.

Ideally, estimates of cancer risk from human travel in space would be based on a mechanistic understanding of complex effects elicited by different types of radiation exposure. Because most adult human solid tumors are epithelial in origin and because radiation effects are dictated by cellular genotype and phenotype, studies of HZE radiation biology using a physiologically relevant model will be most informative regarding risk. Cultured human epithelial cells also permit study of phenotypes and pathways that may be uniquely human or epithelial.

We have developed methods for culturing human mammary epithelial cells (HMEC) derived from surgically discarded phenotypically normal tissue. HMEC can be grown on tissue-culture plastic as traditional monolayers (2D) or in a physiological extracellular matrix as a multicellular (3D) culture. Preliminary data comparing global gene expression in 2D versus 3D HMEC cultures show differential expression of genes involved in DNA damage sensing and repair, in cell death and survival, and in cell cycle regulation. We propose that understanding how controlled cellular and microenvironment conditions affect early responses to densely versus sparsely ionizing radiation-induced DNA damage in terms of repair, foci formation and gene expression profiling can provide the necessary bridge to extrapolating from in vitro to in vivo responses.

Although cancer is considered the major late health risk following low fluence particle radiation exposure, it has a latency of years that is both difficult to track and poorly understood. The use of cultured human cells permits study of radiation-induced phenotypes and pathways that precede neoplasia. Preliminary data demonstrate that sparsely ionizing radiation can elicit cell behaviors, such as loss of multicellular organization, strongly associated with epithelial cancer and deregulation of differentiation. Importantly, the irradiated phenotype is expressed in the progeny of irradiated HMEC.

The phenotype most commonly associated with human cancers is the capacity for unlimited cell proliferation. It is clear that human cells have developed extremely stringent mechanisms to prevent immortal transformation, as exemplified by repression of telomerase, presumably as a mechanism for tumor suppression. When critically short telomeres develop, HMEC that retain wild-type p53 function begin a process called agonescence. The ensuing telomere dysfunction results in chromosomal aberrations, particularly telomere associations, which can lead to growth arrest or mitotic failures. Telomere dysfunction serves as a barrier to immortality and therefore cancer. However an as yet ill-defined combination of genetic and epigenetic changes can lead to derepression of telomerase, culminating in production of infinite lifespan cells. If we determine that radiation exposure alters the stringency of finite lifespan mechanisms, then that could in turn contribute to carcinogenesis. Recent reports suggest that densely ionizing radiation is more efficient than sparsely ionizing radiation in inducing genomic instability, which may contribute to its carcinogenic effect. We will determine the frequency of HZE charged particle radiation induces genomic instability in both finite lifespan HMEC and immortal HMEC. Together, these surrogate functional HMEC endpoints will enable determination of the RBE of HZE neoplastic potential.

These data will be integrated at two levels: by theoretical modeling of the physical events leading to DNA damage and by systems biology modeling of critical pathways. The intent of this 5-year project is to provide a comprehensive picture of HZE effects from the initial damage, to early cellular HMEC responses, to persistent functional precursors of carcinogenesis. NASA areas of interest that will be addressed include: mechanisms, nature, and frequency of DNA damaging events; mechanisms of DNA repair and misrepair, early signal transduction mechanisms; immediate and long-term, and reversible and irreversible gene expression changes; cellular remodeling and reorganization; potential mechanisms of tissue repair and matrix effects; induction and regulation of genomic instability; cellular and molecular mechanisms of charged particle-induced progression to a neoplastic phenotype.

This NSCOR builds on two strong pillars: the significant publication record of the Director, Associate-Director and Team Leaders in HZE radiation biology, and the unique expertise of the Director and Team Leads in normal and malignant breast biology. Participants in this NSCOR have developed and implemented unique cell culture methodologies that more accurately reflect the behavior of human epithelial cells in vivo, and this approach will be integrated with theoretical modeling and systems biology to provide an innovative program to assess HZE particle radiation damage and repair. The use of well characterized human epithelial cells and functional assays of neoplastic behavior make the approach relevant to human health risk estimates. Ultimately, this work will help NASA develop a basic understanding of the mechanisms of HZE radiation damage and repair and their contributions to the neoplastic process.